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6) Hypercarbia and hypoxaemia secondary to the pneumoperitoneum, factors known to produce heightened glucose response during surgery (Ortega et al, 1996; Hendolin et al, 2000; Luo et al, 2003; Koivusalo et al, 1998; Coskun et al, 2000) cannot be regarded as the possible causal factors because these two complications did not occur in any of the patients studied in the present series.

7) The adverse influence of wound pain on respiration with resultant hypoxaemia cannot be ignored as it is known to produce heightened glycemic response (Chumillas et al, 1998; Ali and Gana, 1998; and Larsen et al, 2002) and it has more pronounced and long-lasting impact on patients’ convalescence in open cholecystectomy than in laparoscopic surgery. This may be the reason why the intergroup difference in the glycemic response was found significant (<0.001) on the 1st and 4th postoperative day between the both the lap and open groups and insignificant (>0.05) between the two open groups (OC vs. OH).

8) The intergroup difference between OC and OH was found insignificant, indicating equal metabolic stress response in the two open groups.

9) The intergroup difference between laparoscopic and open surgery groups was found highly significant at all the four end-points following start of the operation (p<0.001), indicating stronger and longer lasting glycaemic response following open surgery in the present study.

6.2.2. Serum Cortisol Response

1) In LC group, serum cortisol level increased significantly at half-an-hour of surgery (p<0.001) and peaked at the end of surgery with approximately 167 % enhancement (p<0.001), indicating that even straightforward easy lap cholecystectomy is also a stressful procedure.

2) The high intra-op cortisol level in LC group decreased to near preoperative value on 1st postoperative day (p>0.05), indicating complete normalization from the surgical stress after lap chole.

3) The short-lived accelerated cortisol response during the intra-operative period in the LC group with rapid normalization to the pre-operative level on the very 1st post-op day is in full agreement with the observations of other investigators (Joris et al, 1992; Mealy et al, 1992; Redmond et al, 1992).
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4) The peritoneal stretching during laparoscopy seems to be the important contributory factor for accentuated hormonal (and metabolic) response with stimuli for the stress response arising from visceral and peritoneal afferent nerve fibres in addition to those from the abdominal wall as emphasized by Rademaker and colleagues (1994), Desborough (2000) and Adhikary & Korula (2004).

5) In the LC group, the cortisol concentration on 4th postoperative day was recorded significantly less than the preoperative value (p<0.001), suggesting presence of significant degree of pre-operative stress due to marked anxiety and apprehension, especially in relation to the newer technology and key-holes of lap chole.

6) This also indicates that sampling immediately prior to induction of anaesthesia and surgery is not really representative of the base line concentration of the bio-marker.

7) In OC group, increased cortisol concentration during the intra-operative period also peaked at the end of open surgery with an approximately 229% enhancement (p<0.001), indicating greater stress following open cholecystectomy as compared to the lap chole.

8) The high intra-operative cortisol concentration in the OC group did not return to the preoperative level even on the 4th postoperative day (p<0.001) as compared to the LC group, suggesting continued surgical stress in the late post-op period after open chole.

9) The inter-group difference between intra-op cortisol responses of the LC and OC groups in the present study was statistically significant (p<0.05) with lesser degrees of cortisol response following lap cholecystectomy in accordance with findings of Glaser et al (1995) and Haq et al (2004).

10) The inter-group difference between post-operative cortisol responses of the LC and OC groups in the present study was statistically significant (p<0.001) with markedly lesser cortisol response following lap cholecystectomy as compared to open cholecystectomy in accordance with the findings of Luo et al (2003), Karayiannakis et al (2005) and Crema et al (2005).
11) In the OH group, cortisol concentration significantly increased during the intra-operative period and peaked at the end of surgery with an approximately 282% enhancement (p<0.001) in consonance with the finding of Moore et al (1994).

12) The high intra-operative cortisol concentration in OH group did not return to the preoperative level even on 4th postoperative day (p<0.001) - a finding at variance with that of Moore et al (1994).

13) Inter-group difference between the LC and OH groups were statistically significant not only during the intra-operative period but also during the postoperative period (p<0.05 at half-an-hour and p<0.001 at other time points), with markedly lesser cortisol response following lap chole.

14) Inter-group difference between the OC and OH groups was not significant statistically (p>0.05) at all the time points, indicating the similar stress responses in the two open groups in terms of serum cortisol changes.

6.2.3. Serum Epinephrine Response

1) In LC group of the present study, there was slow intraoperative rise in epinephrine concentration with peaking at the end of surgery (~2-fold increase at half an hour and ~3-fold increase at the end of surgery) and then it decreased markedly on the 1st post-op day with only 1.3-fold increase though still different statistically from the pre-op value (<0.05) in full agreement with the observations of Ratge et al (1990), Anand and Hickey (1992), Glaser et al, (1995) and Ortega et al (1996).

2) That means, laparoscopic cholecystectomy was associated with marked intra-operative systemic stress response and significant stress was continued in the post-operative period until 1st post-op day.

3) On 4th postoperative day in the LC group, the epinephrine concentration decreased further and was recorded 17 % lower than the preoperative value, indicating presence of pre-op stress in these patients most probably secondary to the procedure-related anxiety and apprehension, mainly in relation to newer technology as seen also in relation to the cortisol response in the present study.
4) In the OC group, accentuated intra-operative epinephrine response with peaking at the end of surgery (~2.5 fold increase at half an hour after incision and >3.5-fold increase at end of surgery) was recorded and then it decreased slightly but persisted significantly high (>2.5-fold) on the 1st post-op day (p<0.001), indicating marked systemic stress response not only in the intraoperative period but also in the post-operative period until 1st post-op day.

5) On the 4th post-op day in the OC group, the plasma epinephrine level decreased markedly and was found only 5 % lower than the pre-op value, suggesting again the presence of at least some degrees of pre-op stress in these patients possibly secondary to the procedure-related anxiety and apprehension.

6) In spite of intense pneumoperitoneum and peritoneal stretching during intra-operative period, inter-group difference between epinephrine responses of LC and OC was not significant (p>0.05), indicating similar intra-operative systemic stress responses in the two techniques of cholecystectomy.

7) On the 1st post-op day, inter-group difference between epinephrine responses of LC and OC was significant (p<0.001), indicating markedly higher stress response persisting in the OC group on 1st day as compared to the LC group.

8) On the 4th post-op day, inter-group difference between epinephrine responses of LC and OC was not significant (p>0.05), indicating statistically similar fall in epinephrine concentration below the pre-op level in both LC and OC groups although percentage fall below pre-op level on 4th day seems apparently quite different between the two groups (17 % in LC group versus 5 % in OC group).

9) In OH group of the present study, the epinephrine response followed the exact pattern of the epinephrine response of open cholecystectomy (OC) group to the similar degrees during both the intra-operative and post-operative periods.

10) In the OH group, there was 2-fold increase at half an hour after incision and 3.5-fold increase at end of surgery and then slight decrease on 1st post-op day with ~2.5-fold increase with respect to the pre-op value, indicating
marked systemic stress response not only in the intraoperative period but also in the post-operative period until 1st post-op day.

11) On the 4th post-op day in the OH group, the plasma epinephrine level decreased markedly and was found 19 % lower than the pre-op value, suggesting the presence of significant degree of pre-op stress in these patients possibly secondary to the procedure-related anxiety and apprehension, especially in relation to removal of uterus.

12) Inter-group difference between epinephrine responses of OC and OH was not significant at any of the time points (p>0.05), indicating similar perioperative systemic stress responses in the two open surgery groups.

13) Inter-group differences between epinephrine responses of LC and OH followed the exact pattern of differences between LC and OC groups at half an hour after incision, at end of surgery 1st and 4th post-op days, indicating similar systemic stress responses in the two groups during intraoperative period and 4th post-op day, and dissimilar responses on 1st post-op day – markedly less in LC group.

14) Significantly high epinephrine concentration in intraoperative period in open group in absence of pneumoperitoneum cannot be explained on the basis of technique of anaesthesia as suggested by Derbyshire and Smith (1984) because similar general anaesthesia was administered in a planned fashion in all the three groups of the present study.

15) Significantly high epinephrine concentration in intraoperative period in open group in absence of pneumoperitoneum was rather, in all probability, the reflection of extensive prolonged visceral tissue dissection which may be true for the open hysterectomy taking longer duration of time in the present study but that does not hold good for the open cholecystectomy in the present series, which was straight forward easy and was carried out within a time frame similar to that of lap cholecystectomy, reflecting almost same amount of visceral tissue dissection in the two biliary groups.
6.2.4. Serum Norepinephrine Response

1) The pattern of the norepinephrine response in the present study was found almost exactly similar to that of the epinephrine response in the intra-operative as well as post-operative period in all the three groups (LC, OC and OH).

2) Significant rapid rise of NE at half-an-hour after start of the surgery and peaking at the end of surgery though comparable among the three groups were possibly due to different causal factors which have been already discussed in detail under the head of epinephrine and cortisol responses in the present study.

3) However, certain small but important differences between the Epinephrine (EPI) and Norepinephrine (NE) Responses in LC, OC, and OH Groups deserve consideration.

4) In the LC group, there was 2-fold increase in intra-operative NE concentration as compared to the 3-fold increase in intra-operative EPI concentration; however their statistical significance with respect to their pre-operative levels was found similar (p<0.001).

5) In both the open groups (OC & OH), intra-operative NE enhancement (2-fold) was lower than the intra-operative EPI enhancement (3.6-fold) though both values were highly significant with respect to their pre-operative values (p<0.001).

6) **1st post-operative day:** Comparison between LC and open groups (OC/ OH) shows differential NE fall on the 1st post-operative day from the intra-operative peak in the two groups – 43.4 % in LC group and only ~7 % in the open groups. *This observation suggests much faster recovery of the acute stress response in patients operated laparoscopically, lending strong support for the fast evolving Day Case Surgery/ Fast-Track Surgery especially in relation to the laparoscopic procedures.*

7) **On 4th post-operative day,** LC group recorded 15 % fall of NE below the pre-operative value which was statistically significant (p<0.001) while the open groups showed 7 – 13 % fall of NE below the pre-operative level which was not found significant (p>0.05). However, the Inter-group
difference between lap and open groups was not significant statistically (p>0.05).

8) This fall of NE values in the late post-operative period well below the pre-operative levels in all the three groups (LC, OC and OH) of the present study is similar to the changes of epinephrine and cortisol concentration of the present study, further supporting the presence of marked degrees of pre-operative anxiety and apprehension which are potent activators of the sympatho-adrenal system and has been discussed in detail under the of cortisol response. Norepinephrine in the plasma comes mainly from the overflow of the norepinephrine released from the sympathetic nerve terminals (Cryer, 1976).

6.2.5. Serum C - Reactive Protein Response


2) OC group (n=21) recorded accentuated CRP expression soon after start of surgery that persisted until 4th post-op, with peak effect on the 1st post-op day in accordance with the observations of McMahon et al (1993), Redmond et al (1994) and Bruce et al (1999).

3) Intergroup difference between lap cholecystectomy and open cholecystectomy was found statistically not significant, suggesting that both the procedures are equally stressful with respect to acute phase protein response when intra-cavitatory management and visceral tissue dissection is comparable.

4) Open abdominal hysterectomy was followed by intra-operative (intra-op) as well as post-operative accentuation of CRP expression - a finding at variance with only post-operative enhancement of CRP recorded by Moore et al (1994).

5) Pattern of CRP changes following open abdominal hysterectomy was almost similar to that after laparoscopic cholecystectomy in the intra-op
period but not in the post-op period – the classical time for the CRP expression; the most probable cause for this variation in the present study is the longer duration of time taken for the open hysterectomy, indirectly referring to the significance of the visceral tissue dissectional trauma again.

6) A comparison of biliary and non-biliary surgery with respect to CRP expression is reported first time in the present study.

7) Pattern of CRP changes following open abdominal hysterectomy was found almost similar to that after open cholecystectomy but CRP expression after open hysterectomy was more pronounced in the post-op period as compared the open cholecystectomy. This is possibly due to longer duration surgery and visceral dissection in the OH group.

8) Present study confirms the finding of Hill and associates (1995) that neither acute parietal tissue incisional injury nor the pneumoperitoneum affects the CRP expression, indirectly indicating the role of visceral tissue dissection for immune modulation.

9) Present study confirms that lap cholecystectomy does not produce lesser degree of acute phase protein response than the open cholecystectomy or even open hysterectomy.

10) Higher CRP values in the intra-op period as well as post-op period until 4th day indicate a major inflammatory response to the operative procedure done by either open or laparoscopic technique, and reflect perioperative tissue damage and antigen load. Therefore, CRP can be used to quantify the surgical trauma and stress experienced by the individual patients.

6.2.6. Serum TNF-alpha Response

1) LC group (n=21) recorded no enhancement of TNF-alpha expression during intra-op period as well as on the 1st post-op day; however, an insignificant TNF rise was detected on 4th post-op day.

2) OC group (n=21) recorded no enhancement of TNF-alpha expression during intra-op period as in the LC group but developed significant enhancement of TNF-alpha expression on 1st post-op day, with further increase on the 4th post-op day.
3) OH group (n=21) recorded TNF-alpha expression in a manner exactly similar to that seen in LC group.

4) Thus TNF-α showed a characteristic pattern of delayed rise in the post-op period which reached its peak value on 4th postoperative day, and the increased TNF-α production was more marked in open groups (open cholecystectomy and open hysterectomy) as compared to the laparoscopic group, although the inter-group difference was not found statistically significant (>0.05).

5) Thus Lap Cholecystectomy was found as stressful as Open Cholecystectomy or Open Hysterectomy.

6) OC group recorded one exceptionally high value of TNF-alpha on 4th post-op day in 1 out of 21 patients. When this abnormal reading was excluded, time course pattern revealed statistically significant enhancement of TNF-alpha expression at half an hour after incision (p<0.5) as well as on 4th post-op day (p<0.001). However, comparison of this significant time course (n=20) with the LC group (n=21) did not reveal statistical inter-group difference (p>0.05) at any of the five end points.

7) Thus an unexplained exceptionally abnormal reading in the present study did not affect the overall results of TNF-alpha expression in the OC group, validating the adequacy of the sample size for each group of the present study.

8) Thus the similar intra-op acute phase responses in the demographically and technically comparable lap and open groups reflecting equal visceral tissue dissectional trauma in the present study confirms that equal visceral tissue dissectional trauma is the sole or at least major determinant of surgical stress and difference in the parietal tissue injury with respect to the total wound size (2 cms in LC vs. 6-9 cms in OC) did not really matter.
6.3. HAEMODYNAMIC RESPONSE

6.3.1. Heart Rate Response

1) In the LC combined group (n=21), heart rate increased rapidly half an hour after incision (14% increase) and then decreased at the end of surgery after deflation of pneumoperitoneum (7% increase as compared to the preop heart rate).

2) The heart rate practically normalized on 1st postoperative day onward (only 2 percent change).

3) In normotensive patients (n=16) of the LC group, heart rate increased significantly at half an hour after start of surgery (9.4 % increase; p=0.002), at the end of surgery (4.7% increase; p<0.05) and also on the 1st postoperative day (3.52 % increase; p<0.05), and it normalized only on the 4th post operative day (2.35 % increase; p>0.05). This is in consonance with the findings of Lentschener & Benhamou (1996) and Longhurst et al (1981).

4) In the treated hypertensive patients (n=5) of the LC group, heart rate markedly increased at half an hour after incision (23.5 % as compared to 9.4% in normotensive patients; p=0.001) and following deflation of pneumoperitoneum at the end of surgery (10.20% vs. 4.7% in normotensive patients; p=0.003). That means/in this way, heart rate response in treated hypertensive patients of LC group was remarkably different from that of normotensive patients of the same LC group.

5) Heart rate changes in the postop period were small in both the subgroups of LC but they were found significantly different from each other on the 1st postop day (p<0.05).

6) Earlier normalization of HR on the very 1st post-operative day as compared to normotensive patients may possibly be a reflection of necessary chronic medication in the treated hypertensive patients as explained by Danzing and associates (2005).
7) In the combined OC group of the present study, significant intra-operative tachycardia with peak at the end of surgery (12 % increase; \( p<0.001 \)) was followed by normalization of heart rate on the 1st and 4th post-operative days. In both normotensive and hypertensive sub-groups of OC, heart rate alterations in the intra-operative and post-operative phases were found similar as those seen in the Combined OC group, and the inter-subgroup differences were statistically insignificant at all the end points (\( p>0.05 \)).

8) HR changes after open cholecystectomy (OC) were found almost similar to those observed after lap cholecystectomy (LC), with no significant inter-group differences at any of the five end points (\( p>0.05 \)). However, slightly different patterns of change in the two groups need a little attention.

9) In LC group, peaking occurred immediately after start of the procedure while peak in OC group was achieved at the completion of the surgery. This suggests that acute rise in the intra-abdominal pressure, that is, pneumoperitoneum produces much more adrenergic stimulation than the surgical procedure itself but does not have an additive effect at the end of operation.

10) The intra-operative change in heart rate noted in treated hypertensive patients undergoing laparoscopic cholecystectomy (LC) was found considerably higher as compared to that seen in the treated hypertensive patients undergoing Open cholecystectomy (\( p<0.001 \)), suggesting again the impact of high pressure pneumoperitoneum.

11) OH group exhibited heart rate changes in a fashion almost similar to those seen in the other open surgery group, that is, OC group – significant intra-operative tachycardia with peak at the end of the procedure and post-operative normocardia on 1st day onward. This confirms that similar adrenergic stimulation was exhibited not only after lower but also upper abdominal open surgery.

12) Inter-subgroup difference between the heart rate responses of the normotensive (n=15) and hypertensive (n=6) patients who underwent open hysterectomy was not significant at any of the five end points (\( p>0.05 \)). This inter-subgroup difference in OH group is exactly similar to that seen in the OC group but dissimilar to that of LC group.
3) In OC group, all variables of arterial blood pressure (systolic, diastolic and mean) were increased significantly during intra-operative period with minor differences at half-an-hour after incision and end of surgery, and all of them returned to near or even below the pre-operative level on the very 1st postop day.

4) These observations of OC group are at a little variance to those seen in the LC group where rapid peaking effect at half-an-hour was noted in all the variables of arterial blood pressure (systolic, diastolic and mean).

5) This implies that both open and lap cholecystectomy procedures are equally stressful with similar intra-cavitatory management with only one difference at half-an-hour after incision and that was due to additional mechanical effect of the pneumoperitoneum during lap cholecystectomy which was not found to be additive in the subsequent part of the procedure.

6) In the OH group, all variables of arterial blood pressure (systolic, diastolic and mean) were increased significantly during intra-operative period and their normalization took place on the very 1st post-operative day. However, it is interesting to note that systolic and mean arterial blood pressure achieved peak at half-an-hour after incision while heart rate and RPP showed peaking effect at the end of the surgery; however minimal changes were seen in the diastolic blood pressure.

7) The significant intra-operative increases in the arterial blood pressures (systolic, diastolic and mean) observed in the present study were accompanied by the significant intra-operative increases in plasma epinephrine and norepinephrine concentrations in the present study.

8) Intra-operative increase in arterial blood pressures and plasma concentrations were individually highly significant but the time course pattern was found different: peaking of the arterial blood pressures (systolic, diastolic and mean) occurred at half-an-hour after incision while plasma catecholamines achieved peak at the end of surgery, suggesting interplay of additional factor(s).

9) The most probable factors for maximal haemodynamic changes soon after creation of the pneumoperitoneum include pneumoperitoneum-induced compression of intra-abdominal great vessels as emphasized by majority of investigators or direct sympathetic nervous system stimulation as emphasized by Katz and Bigger (1970), Orloff (1981) and Haleem et al (1991) or

10) In OC group, correlation of arterial blood pressure changes and plasma catecholamine changes seems good (Table 4-220). Therefore, the causal mechanisms for these changes may include peritoneal stretching and stimulations in open surgery causing vasopressin release as emphasized by Lentschener et al (2001), or direct stimulation of sympathetic nervous system as emphasized by Katz and Bigger (1970), Orloff (1981) and Haleem et al (1991), or increased sympathetic tone resulting from reflex activity (Marshall et al, 1972).

11) In OH group, correlation of arterial blood pressure changes and plasma catecholamine changes does not hold good. Hence, role of factors involved in the open surgery as discussed in the OC group assumes importance for causation of equally significant alterations in arterial blood pressure and other haemodynamic parameters.

6.3.3. Rate-Pressure Product

1) RPP, a major determinant of myocardial oxygen consumption, increased during the intra-operative period in all the three groups and decreased markedly to lower than the pre-op value.

2) LC (combined) group showed maximum intra-operative increase in RPP with rapid peaking (29.6 % increase) at half an hour after start of surgery and then post-operative decrease to lower than the pre-op value by 8.5-9.5 %.

3) OC (combined) group revealed persistently high RPP (21 - 23 % increase) throughout the procedure and then experienced post-operative normalization to the pre-op value (<3 % difference).

4) OH (combined) group showed gradual intra-operative rise in RPP with a maximum of 14 % increase at the end of surgery and then postoperative decrease to lower than the pre-op value by 4-5.5 %.

5) Hypertensive patients in all the three groups (LC, OC & OH) experienced greater intra-operative increase in RPP than Normotensive patients with rapid
maximum rise at half an hour after incision and post-operative decrease to lower than the pre-op value by 4 - 9.5 %.

6) LC-Normotensive subgroup showed intra-operative increase in RPP with maximum rise at half an hour after incision in a fashion similar to LC-Hypertensive subgroup but to a lesser degree and post-operative decrease to lower than the pre-op value by 8.6 - 9.5 %.

7) OC-Normotensive subgroup revealed persistently high RPP throughout the intra-operative period and complete normalization post-operatively to the pre-op value.

8) OH-Normotensive subgroup showed minimal rise (<2 %) at half an hour after incision and mild increase at the end of surgery (11 %) and then post-operative decrease to lower than the pre-op value by 7 %.

9) Dangerous level of RPP was crossed only in the Hypertensive patients undergoing laparoscopic cholecystectomy soon after start of surgery (half an hour), suggesting real high risk in these patients.

10) Mild RPP increase (8 %) persisting even on the 1st post-op day in Hypertensive patients of LC group in absence of complication indicates continued higher myocardial oxygen consumption even after 24 hours of surgery that is apparently correlated to the high pressure carpopneumoperitoneum.

11) All subgroups except OC-Normotensive experienced 4 -9.5 % fall below the pre-op value on the 4th post-op day, indicating presence of significant degree of pre-operative mental stress due to surgery-related anxiety and apprehension, especially with respect to the newer technology or removal of uterus.

12) In nutshell, by standardizing and minimizing peri-operative physiological, pharmacological and pathological variables in the present prospective randomized control clinical trial, we have demonstrated specific surgical procedure and surgical site related changes in stress biomarkers. We assessed the role of blood glucose, cortisol, epinephrine, norepinephrine, CRP, TNF-α levels as tentative stress markers in correlating the extent of perioperative metabolic, hormonal changes and immunomodulation during open and laparoscopic cholecystectomy and abdominal hysterectomy (as a control) in a subset of an Indian population.
13) Finally, we correlated the stress biomarkers findings with hemodynamic parameters recorded on the time of blood sampling. We found cholecystectomy and hysterectomy are stressful procedures which modify the stress response significantly, either performed through open incision or laparoscopically.

14) Following laparoscopic surgery, the stress hormonal responses were more pronounced during intraoperatively period. However, in the postoperative period catabolic hormonal responses were more obvious and continued after abdominal hysterectomy and open cholecystectomy.

15) The hypothesis that a laparoscopic cholecystectomy results in less operative stress is only partly substantiated by analysis of plasma cortisol, plasma glucose, epinephrine and norepinephrine, CRP and TNF-α responses to surgery-induced injury. As advocated by Hayes et.al (1996), we would caution against over enthusiastic ambulatory laparoscopic cholecystectomy on the rational but unproved assumption that early discharge will lead to occasional delay in diagnosis and management of long term complications.

16) The society of American Gastrointestinal and Endoscopic Surgeon (SAGES) 2005 Guidelines assume greater significance in view of present study as better surgical skill will minimize the visceral tissue dissection Trauma with attenuation of various acute phase responses that are deciding factor for the final outcome as evident by perioperative modulation of TNF-α and CRP. However, this also reflects that these selective stress markers have a limited role in the clinical assessment of immune stress responses and tissue trauma. Our finding warrants the search for more sensitive biomarkers for peri and postoperative management of surgical patients.

17) The insult associated with pneumoperitoneum cannot be under scored specially in high risk cardiopulmonary disease patients (Wittgen et al, 1991).